Cytology of Some California Grasshoppers. 1. Taxonomic Considerations

(Orthoptera:Acridoidea)

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The purpose of this paper is to call attention to the extent of chromosomal variation, both structural and numerical, in several species of grasshoppers and its bearing on orthopteran taxonomy.

Many grasshoppers have evolved novel genetic systems which are not only of intrinsic interest to evolutionary biologists but in many cases can be taxonomically useful (White, 1951, 1954). During the course of studying the effects of chromosome rearrangements on genetic recombination in various species (Hewitt, 1967; Hewitt and Schroeter, 1968), numerous other species were collected in California which had not been previously studied cytologically. The taxonomic status of some of these has remained in doubt due to the fact that morphological features considered alone have not proved reliable enough in establishing phylogenetic relationships. Six species are reported on here which make clear that a chromosomal analysis could, indeed should, be used in any serious taxonomic treatment.

MATERIALS AND METHODS.—The species discussed, sites and dates of collection, and number of individuals examined cytologically are given in Table 1.

Testes from adult male grasshoppers were removed following a mid-dorsal incision and immediately placed in a fixative of 3:1 absolute ethyl alcohol: glacial acetic acid. Ovarioles of females were removed by vivisection under insect saline and then cultured for 30 minutes in 0.05% colchicine in insect saline to arrest and accumulate mitotic metaphases before being similarly fixed. A detailed description of the reproductive anatomy of grasshoppers is to be found on pages 138–150 in Uvarov (1966).

In our experience young male imagines are a good source of meiotic material but meiotic divisions can usually be found even in the testes of males collected late in the season. Only young female imagines have proved to be satisfactory for the study of ovariole wall mitosis.

Following fixation the material was stored in the fixative or 70% ethanol in a refrigerator and subsequently squashed in acetic-orcein.

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TABLE 1. Data on the six species examined cytologically in the present study.

Species	Collection site	Date collected	Number examined cytologically
Tanaoceridae			
Tanaocerus koebelei Bruner	15 mi. W. Panamint Springs, Inyo Co.	7 May 1966	9 ♀♀
Acrididae			
Romaleinae			
Dracotettix monstrosus Bruner	Pope Valley, Napa Co.	15 June 1966	1 ♂
Cyrtacanthacridinae			
Oedaleonotus borckii (Stal)	Jackson, Amador Co.	3 July 1966	8 ♂♂
	Telephone Campground, Glenn Co.	13 Aug. 1966	12 "
Oedaleonotus enigma (Scudder)	15 mi. S. Mendota, Fresno Co.	28 May 1966	26 "
	1.0 mi. E. Oilfields, Fresno Co.	28 May 1966	27 "
	16 mi. S.W. Five Points, Fresno Co.	28 May 1966	99 "
	" " " " " " "	5 Aug. 1968	216 "
	2 mi. E. Oilfields, Fresno Co.	28 May 1966	86 "
	" " " " " "	20 May 1967	29 "
	" " " " " "	8 June 1967	106 "
	" " " " " "	2 July 1967	63 "
	" " " " " "	28 July 1967	25 "
	" " " " " "	3 Aug. 1968	43 "
	13 mi. N. Coalinga, Fresno Co.	28 May 1966	111 "
	" " " " " "	9 June 1967	98 "
	" " " " " "	2 July 1967	88 "
	" " " " " "	28 July 1967	15 "
	8 mi. E. Coalinga, Fresno Co.	28 May 1966	46 "
Oedaleonotus orientis Hebard	About 3 mi. E. Sonora Pass Summit, Mono Co.	1 July 1966	2 "
Oedaleonotus phryneicus Hebard	Los Osos, San Luis Obispo Co.	18 Aug. 1966	3 "
	" " " " " "	31 July 1967	93 "

For details concerning stain preparation and the squash technique the reader is referred to pages 199–205 in Lewis and John (1964).

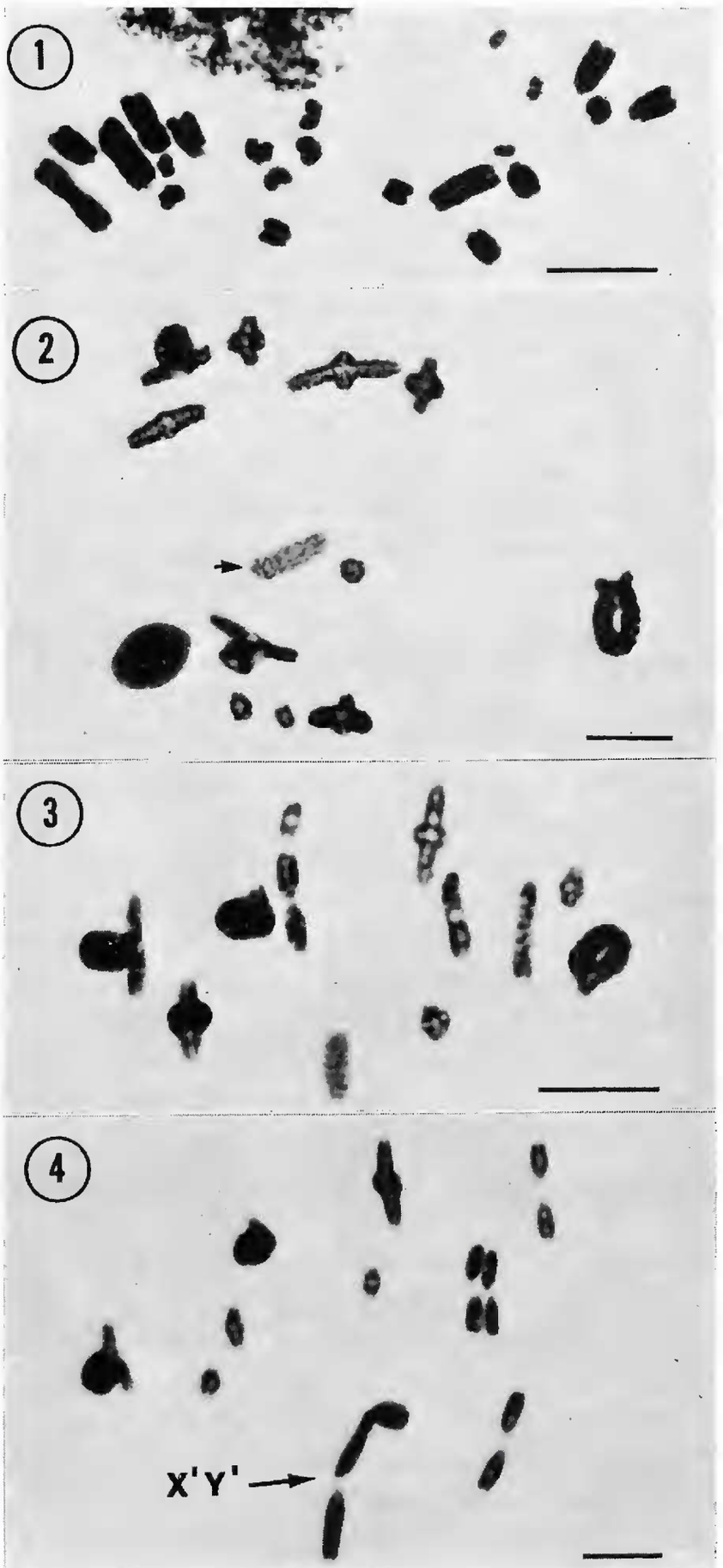
Cytological Observations and Discussion

Tanaocerus koebelei Bruner.—The family Tanaoceridae contains but three species, two of which are found in California. The relationship of these desert longhorns to the rest of the Acridoidea remains obscure. In examining the proventricular region of the tanaocerids and comparing it with other grasshoppers, Grant and Rentz (1967) concluded that the tanaocerids are quite different from both the acridids and eumastacids. On the other hand, Dirsh (1956, 1961) considers the Tanaoceridae to be more allied to the eumastacids and includes them in the Eumastacoid section of his taxonomic scheme which is based upon comparative studies of the phallic complex. It should be pointed out that only the epiphallus of *Tanaocerus koebelei* was studied, a structure considered by Dirsh (1956) to be a taxonomic character reliable enough for differentiating families or even groups of families in some cases.

Figure 1 shows an ovariole colchicine-arrested (c-mitotic) metaphase with 22 acrocentric chromosomes. In the past the term "acrocentric" has been used to describe chromosomes with visible short arms and also those in which short arms were not able to be resolved with the light microscope but presumed to be present. This is discussed by John and Hewitt (1968) and White (1969). To avoid confusion, the term acrocentric will be used in this paper in its broad sense, realizing that rod-shaped chromosomes in many instances may possess truly terminal centromeres (i.e., are telocentric). Until a male individual of *Tanaocerus* is studied, a decision concerning the sex-determining mechanism cannot be made. However, if it is the same as in most other grasshoppers, it is an XO♂/XX♀ system, with the X in this case also being acrocentric.

Hundreds of acridid species have been studied and most have a karyotype consisting of $2n♂ = 23$ ($2n♀ = 24$) acrocentric chromosomes (White, 1969). Although fusions between chromosomes have occurred in many species, thus reducing the chromosome number, this does not explain the peculiar karyotype of *Tanaocerus koebelei* in which all the chromosomes are acrocentric. A reduction in number from 22 to 20 by "centric fusion" would be expected to result in a pair of chromosomes with median or sub-median centromeres.

On the other hand, the chromosome complement of *Tanaocerus koebelei* graded in size is very similar to what could be argued as the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X'Y'

basic karyotype in the Eumastacidae. It closely resembles that of the Pseudoschmidtiinae and Mastacideinae subfamilies, the cytogenetics of which have been described by White (1970a). As his study makes clear, however, the eumastacids are chromosomally quite varied and a morphologically diverse group.

Certainly more information about the cytogenetics of these groups needs to be obtained before any conclusion concerning their phylogenetic interrelationships can be made.

Dracotettix monstrosus Bruner.—White (1954) has drawn attention to the discussion as to whether the Old World subfamily Pamphaginae is represented in the New World by such genera as *Phrynotettix* and *Dracotettix*, or whether these belong in the subfamily Romaleinae. He points out that the Old World Pamphaginae have 21 (20 + X) chromosomes in males while the Romaleinae possess 23 (22 + X). Since *Phrynotettix* males also have 22 + X chromosomes (McClung, 1914) its inclusion in the latter subfamily seems warranted. Consequently we have determined the basic karyotype of *Dracotettix monstrosus* and found a male to have 22 + X acrocentric chromosomes (Fig. 2). This clearly indicates that its inclusion in the Romaleinae is warranted and both cases together argue against the presence of the Pamphaginae in the New World.

Oedaleonotus borckii (Stal).—A total of 20 individuals from two populations on either side of the Central Valley of California has been examined and both samples appear to be cytologically uniform with males having 22 + X acrocentric chromosomes (Fig. 3).

Oedaleonotus enigma (Scudder).—Over 1,000 males of this species have been collected in the lower San Joaquin Valley near Coalinga. This particular species exhibits considerable chromosome variation and differs strikingly from other members of the genus so far studied in its sex-determining mechanism (Hewitt and Schroeter, 1968). In contrast to the XO♂/XX♀ sex-chromosome systems common to most

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FIG. 1. Mitotic complement of *Tanaocerus koebelei*. 2n♀ = 22. Note the terminal or near-terminal position of the centromeres. × 1125. FIG. 2. First meiotic metaphase of *Dracotettix monstrosus*. 2n♂ = 23. Eleven bivalents and a single unpaired sex chromosome (arrow). × 925. FIG. 3. First meiotic metaphase of *Oedaleonotus borckii*. 2n♂ = 23. All chromosomes possess terminal or near terminal centromeres, including the X chromosome. × 1250. FIG. 4. First meiotic metaphase of *Oedaleonotus enigma* with the 'basic' karyotype of 2n♂ = 22. Note the neo-XY sex bivalent (X'Y'). All chromosomes are acro-(telo-?) centric except the neo-X. × 850. (Line scale = 10μ.)

grasshoppers, *O. enigma* possesses a neo-XY (X′Y′) ♂/Neo-XX (X′X′) ♀ mechanism which has evolved from the former by the fusion of the heterochromatic X with an autosome to form the Neo-X, the homologous autosome becoming the Neo-Y. Instead of possessing an unpaired X-chromosome, males of this species are characterized by having a heteromorphic sex bivalent at the first meiotic metaphase composed of a metacentric Neo-X and an acrocentric Neo-Y (Fig. 4). The few females which have been examined possessed two Neo-X's. Thus, instead of males having 23 (22 autosomes + X), both males and females of this species have 22, 20 autosomes plus the two sex chromosomes.

While 2n = 22 is considered to be the basic chromosome number for this species, male individuals have been found in all populations so far sampled with 20 and 21 chromosomes. This variation in chromosome number is due to the fact that *O. enigma* is polymorphic for a centric fusion involving two non-homologous acrocentric chromosomes, numbers 4 and 5. A fusion between these two chromosomes has occurred at or near their centromeric regions resulting in one large metacentric chromosome. An individual may (i) possess the basic karyotype of 20 acrocentric autosomes, (ii) be heterozygous for the fused and unfused chromosomes or, (iii) be homozygous for the two fused metacentric chromosomes. Those individuals which are heterozygous for the fusion possess nine chromosome pairs and a V-shaped chain-of-three at M1 of meiosis (Fig. 5), while those homozygous for the fusion have 10 pairs, one of which is a large ring bivalent made up of the two fused metacentrics (Fig. 6).

The frequencies of the two types of chromosomes involved in this fusion polymorphism vary from population to population, but no population so far sampled has become fixed for either of the two types, i.e., no monomorphic population has been found. There are, however, differences in frequencies between populations for the fused and unfused chromosomes (Schroeter and Hewitt, in preparation).

Although the Neo-XY sex-determining mechanism and the fusion polymorphism are the most conspicuous cytological features of *O. enigma*, there are several other chromosomal alterations for which the species is also polymorphic.

The first of these is a centromere 'shift' in the third smallest chromosome (no. 8). Whereas all the chromosomes of the basic karyotype are acrocentric, a transposition of the centromere has occurred in the number 8 chromosome presumably by means of a pericentric inversion, resulting in a chromosome with the centromere in a sub-median position. Again, in all samples studied, three types of individuals have been

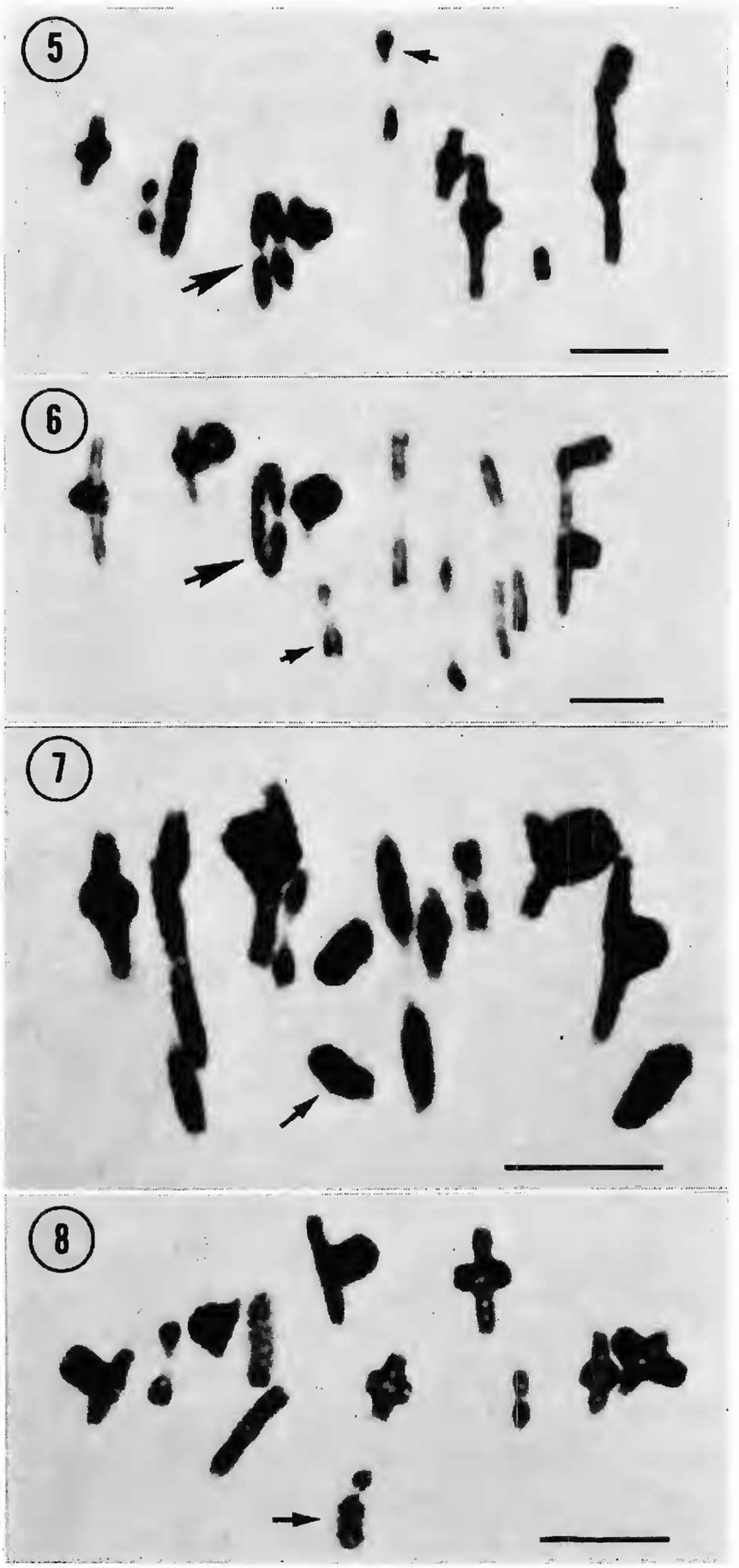
observed: (i) those homozygous for the two acrocentric no. 8 chromosomes; (ii) heterozygous for the acrocentric and submetacentric forming a heteromorphic bivalent (Fig. 5) and, (iii) homozygous for the two submetacentrics.

In some individuals one member of the smallest chromosome pair, no. 10, has an additional heterochromatic segment attached to it, resulting in a conspicuous unequal bivalent at M1 of meiosis (Fig. 6). While this enlarged chromosome has been found in all but one of the populations sampled to date, its frequency is usually so low that only a very few individuals have been found to be homozygous for it.

The last chromosomal variable to be mentioned is that some individuals in some populations possess heterochromatic supernumerary or B-chromosomes which do not pair with any member of the standard chromosome complement. The occurrence of these B-chromosomes is even lower than that of the segment attached to chromosome 10, its frequency not exceeding 2.5% in any of the populations so far sampled. The meiotic behavior of these and the other variable components of the chromosome complement of *O. enigma* and their possible role in the population dynamics of the species have been discussed in a previous paper (Hewitt and Schroeter, 1968). However, it is worth emphasizing that these B's are both metacentric and rare, and consequently, unlikely candidates for the extra sex chromosome arm described in three individuals of this species (Hewitt and Schroeter, 1968) as has been suggested by White (1970b).

Oedaleonotus orientis Hebard.—Only two individuals of this species have been collected, but both exhibited a striking chromosomal feature which may prove to be of considerable taxonomic importance. This is the ditactic bivalent (Fig. 7). This peculiar type of bivalent has been thought to be due to chiasma formation between the short arms of two acrocentric chromosomes, but John and Hewitt (1966, 1968) have interpreted ditactic bivalents to result from chiasma formation within truly terminal centromeres. Whatever interpretation may ultimately be applied, in this instance the ditactic bivalent is a feature that was consistently observed in all M1 cells of both individuals and has not been observed in any of the allied species so far analyzed. As in *O. borckii*, males of this species possess 23 acrocentric chromosomes.

Oedaleonotus phryneicus Hebard.—Only a single population of this species has been sampled to date with a total of 96 males having been examined cytologically. The chromosome complement, typical of most acridids, consists of 22 autosomes and one unpaired sex-chromosome in males, all being rod-shaped with terminal centromeres. There is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one feature which this species shares with *O. enigma*, i.e., a supernumerary chromosome segment attached to a member of the smallest autosomal pair. About 12% of the individuals observed were found to be heterozygous for this segment, resulting in an unequal bivalent as seen in Fig. 8. Each of two individuals also possessed an unpaired heterochromatic supernumerary chromosome.

Four of the seven presently recognized species of *Oedaleonotus* having been studied cytologically, the fact emerges that these four differ in some unique way from each other. *O. enigma* contrasts strikingly with the others by possessing a Neo-XY sex-determining mechanism and being polymorphic for an autosomal centric fusion. *O. orientis* is the only species so far studied in which a ditactic bivalent is formed at M1 of meiosis. *O. phryneicus* differs from *O. enigma* in its sex-determining mechanism (XO♂/XX♀) but is similar to the latter in that it also is polymorphic for a supernumerary chromosome segment and supernumerary chromosomes. *O. borckii* differs from the other three in its being monomorphic, there being no apparent chromosome polymorphism nor any type of peculiar bivalent formation or sex-determining mechanism. Thus the *Oedaleonotus* complex would seem to be a most suitable and extremely rewarding area for cytogenetic and evolutionary investigations.

Based on the studies made, it is clear that cytological techniques can be used to great advantage in a serious taxonomic treatment of this genus. Indeed, we would consider any such study to be lacking if the chromosome complement was not taken into account. Strohecker, *et al.* (1968) state that "satisfactory treatment of the forms of this genus will require comprehensive study not feasible at present. Specimens from widely separated localities appear to represent quite distinct species but many intermediate forms occur and we have not been able to find sets of characters consistent enough to be regarded as of specific worth." It may well be that the chromosome complement will prove to be a worthy character.

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FIG. 5. Metaphase I of *Oedaleonotus enigma* which is heterozygous for the 4–5 fusion forming a chain-of-three (large arrow) and a centric shift in chromosome no. 8 (small arrow). × 1050. FIG. 6. Metaphase I of *Oedaleonotus enigma* which is homozygous for the 4–5 fusion (large arrow) and heterozygous for the supernumerary segment on chromosome no. 10 (small arrow). × 1000. FIG. 7. Metaphase I of *Oedaleonotus orientis*. 2n♂ = 23. Note the ditactic bivalent (arrow). × 1700. FIG. 8. Metaphase I of *Oedaleonotus phryneicus*. 2n♂ = 23. Note the unequal bivalent (arrow). × 1350. (Line scale = 10μ.)

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